KW 39

1. DPN1-digested PCR products on agarose gel. Gel-extraction of Lov and PDZ with Qiagen Gel-extraction Kit
2. PJet blunt ligation of PCR products with overhangs in backbone pmz333.2 according to Qiagen protocol for 2h at room temperature
3. Trafo of pJET plasmid in e.coli according to protocol
4. colony PCR of 16 picked (8 colonies each)

- results: 4 colonies of LOV and PDZ each chosen for overnight cultures plus two blind samples each

5. overnight cultures of pJET-plasmids LOV and PDZ
6. miniprep of pJet overnight cultures according to Qiagen plasmid miniprep protocol
7. XbaI-NotI-HF digest of 4 selected miniprepped colonies (LOV1, LOV3, PDZ1, PDZ 4) for 2h with 20 ul plasmid
8. gel-excision of products: PDZ ~2kb, LOV ~1,4kb and gel-extraction
9. gel.extracted products put on test gel for concentration comparison (for ligation use) to our cut backbone

- approx. ratios:
  LOV1:pmz333.2B = 8:1
  PDZ1:pmz333.2B = 4:1
  LOV3 and PDZ4 are not going to be used

10. Quick ligaion of each LOV1 and PDZ1 with the backbone (see ration above) for ~5 min
11. to be sure (if step 10 may have failed): normal ligation with ligase and T4 ligase buffer
12. Trafo of the ligated LOV1-pmz and PDZ1-pmz in e.coli according to protocol and on agar plate with Amp
13. in total 32 colony PCRs of transformed e.colis: Quick ligation was a success!
14. chosen according to the best colony PCR results, we made 8 overnight cultures of the picked colonies of each product (LOV1-pmz and PDZ-pmz)
15. miniprep of the overnight cultures according to Qiagen-Kit, nanodrop results were equally good
16. 4 test digests for 1h, 37°C:

- LOV1,2,4,5 cut with XbaI and XhoI: desirded DNA lengths 2754bp, 861bp, 604bp were seen
- PDZ1,2,4,5 cut with XhoI: DNA lengths at 3347bp, 1433bp were seen

17. LOV1 and PDZ1 are used further:

- multiplying the rests of step 14th overnight cultures in 100ml LB+Amp